

Experiments on decolourization with 122-resin for extraction of gibberellins from solid medium under different conditions

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Abstract: The extracting liquid of gibberellins (*Gibberella fujikuroi*) from solid medium for was decolorized separately with 75%, 95% alcohol, and distilled water in static adsorption and vibrating way for different durations. The results showed that the content of GA₃ in efflux extracted with alcohol is 10% higher than that with distilled water. With the increase of the durations of extraction, the content of GA₃ increases and the dissolution of pigments also increase. For extraction of GA₃ cultured in solid medium, the best decolorizing result was obtained when it was extracted with 75%-95% alcohol in static way for 1-3 h, or in a vibrating way for 30-60 min, and then to decolor in a static way for 30-60 min in 122 resin column.

Keywords: Gibberellins (GA₃); Solid Culture; Extraction; Adsorption; Decolourization 122-Resin

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Introduction

Gibberellins (*Gibberella fujikuroi*) (GA₃) is a natural plant growth regulator, originated from rice pathogenic microbe. It has been widely studied and developed as a lab and commercial benefit matter for the growth and development of plant in production or in molecular biology, such as induction of activation of hydrolytase in seeds, elongation of stem, induction of flowering, and stimulation of fruit-bearing, (Wang *et al.* 1996; Gu 1999; Wu *et al.* 2001; Advances in molecular Biology for *Gibberella fujikuroi* <http://www.agrisci.com/HrdbBrief>). In the process of post stage of solid culture for, GA₃ a mass of purple pigments are formed (Shanghai Municipal 920 Coordinating Team 1973; Li *et al.* 1992), meantime farm by-product used in culture also contains a lot of pigments. All these pigments bring about difficulties in separation of GA₃ and affect the appearance and quality of the product. Therefore it is very important to decolor these pigments. Model 122-resin is a newly cation adsorption and exchange resin which can adsorb and dispel the pigments in substrate away to purify and separate the GA₃. In this study, experiments on decolorizing with 122-resin are conducted under different conditions to find the best way for extraction of GA₃.

Materials and methods

Model 122 cation resin (Shanghai Chemical College An-

tibiotic Laboratory and Shanghai Zhonghua Pharmaceutical Factory 1989) was made in Yangzhou Pharmaceutical Factory, blood-red granule with diameter of 0.2–1.0 mm, Ph=5.

GA₃ was detected with spectrofluorimetry by Model 930 Spectrofluorimeter (made in Shanghai NO.3 Analysis Instrument Factory), with emitting wavelength of 320 nm and fluorescent wavelength of 420 nm. Assay of turbidness was conducted with Model 721 spectrophotometer (made in Shanghai NO.3 Analysis Instrument Factory).

Extraction of GA₃ in solid medium was separately conducted with 75%, 95% alcohol, and distilled water for 1 h, 3 h, 5 h in a static way and for 10 min, 30 min and 60 min in a vibrating way, respectively; then pour the efflux into the column of 122 resin to decolor the pigments containing in the influx..

The above mentioned efflux was separately decolorized in 122-resin column for 5 min, 10 min, 30 min and 60 min in a static soaking way.

122-resin was soaked into HCl according to the ratio of resin to HCl (1.5–2.0 M) =1:3 (v/v) for 3 h, then soaked into solution of NaOH according to the ratio of resin to NaOH (1.5–2.0M)=1:3(v/v) for 3 h to rinse the residue of HCl, and soaked into HCl for 3 h again. After that the resin was washed thoroughly to neutrality with tap water and distilled water. Then the resin was soaked into 95% alcohol for 3 h and leak to dry. Afterwards the resin was soaked into 1:3 (v/v) alcohol for 2 h, and then washed to free alcohol with distilled water. Finally, the resin was filled to the half of the volume of the column.

Results and analysis

Effect of soaking time in 122-resin on decoloring

Different static time in 122-resin column for GA₃ extrac-

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tion presented, different decoloring results (Table 1). The worst result came into being the treatment for 5 min, which had a light purple appearance and high turbidness (42.5), though the content of GA₃ is as high as 954.7 mg · kg⁻¹.

Table 1. Impact of different soaking time in 122-resin on decolorizing for GA₃ extraction

Soaking time /min	Appearance of efflux	Turbidness	Concentration of GA ₃ /mg · kg ⁻¹
5	Light purple	42.5	954.7
10	Light red	28.6	836.9
30	Nearly free color	2.3	821.2
60	Nearly free color	0.2	819.4

Extraction of GA₃ with different extractants in a static way

The solid medium of *G. fujikuroi* was separately extracted with 75%, 95% alcohol, and distilled water for 1, 3, and 5 h, and then the efflux was poured into 122-resin column to adsorb and decolor in a static way for 60 min. With regard to decoloring, the result of extraction with distilled water was best, its efflux had free color appearance, and turbidness is almost zero even at 5 h extraction. The extraction with 75% alcohol also presented a good result, for which the efflux had free color appearance and turbidness is 0.0, but it turned into slightly light milky appearance with little turbidness at 3 h and 5 h. However, concerning the content of GA₃ in the efflux, it is lowest for extraction with distilled water, and highest for extraction with 95% alcohol (Table 2).

Table 2. Results of extraction of GA₃ in solid medium with different extractants and different static time

Extractants	Static time/h	Appearance of efflux	Turbidness	Concentration of GA ₃ /mg · kg ⁻¹
95% Alcohol	1	Free color	0.1	846.5
	3	Light milk	8.2	857.3
	5	Light milk	15.3	892.4
75% Alcohol	1	Free color	0.0	838.4
	3	Free color	0.05	840.7
	5	Light milk	4.7	849.5
Distilled Water	1	Free color	0.0	780.2
	3	Free color	0.0	785.6
	5	Free color	0.04	801.9

Note: decolorizing with 122-resin column (soaking for 60 min)

Extraction of GA₃ with different extractants in a vibrating way

In this experiment, a vibrating method was adopted to improve the decoloring result. The experimental results showed that vibrating is very effective approach for decolorizing and increasing the content of GA₃. The result for the extraction with 95% alcohol and vibrating for 10–30 min is best. Good decolorizing result was also obtained for extraction with distilled water by vibrating, but the content of GA₃ extracted in the efflux was lower than that of treatment

with alcohol. Meanwhile the longer the duration of decoloring is in vibrating way, the higher the content of GA₃ extracted, but the corresponding appearance looks dark (Table 3).

Table 3. Results of extraction of GA₃ with different extractants and vibrating time

Extractants	Time /min	Appearance of efflux	Turbidness	Concentration of GA ₃ /mg · kg ⁻¹
95% Alcohol	10	Free color	0.06	870.3
	30	Near free color	3.7	874.7
	60	Light red	27.5	908.2
75% Alcohol	10	Free color	0.0	865.8
	30	Free color	1.2	870.3
	60	Slight light red	8.5	885.4
Distilled Water	10	Free color	0.0	802.4
	30	Free color	0.08	810.5
	60	Light brown	10.2	841.5

Note: decolorizing with 122 resin column (soaking for 60 min)

Conclusions and discussions

For extraction of GA₃ cultured in solid medium, the best decoloring result can be obtained when it is extracted with 75%–95% alcohol in static way for 1–3 h, or in a vibrating way for 30–60 min, and then to decolor in a static way for 30–60 min in 122 resin column.

Both decoloring result and the final fact concentration of GA₃ as well as of the time of decolorizing and extraction should be considered for extraction of GA₃. Comparing with alcohol, distilled water dissolves less pigments in decolorizing efflux in spite of extracting in a static way or in a vibrating way, though it is cheaper. The content of GA₃ in efflux extracted with distilled water is 10% less than that extracted with alcohol due to the fact that GA₃ has a lower soluble rate in water than in alcohol. The longer the duration of extraction lasts, the higher the content of GA₃ extracted, and the more the pigments is dissolved.

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